Bell pepper rootstocks with multiple resistance to soilborne diseases

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ABSTRACT

Grafting has been increasingly used to overcome soilborne pathogens. The commercial rootstock hybrids AF-8253 (Sakata) and Fortaleza (Takii), as well as Embrapa's new hybrid, BRS Acará, were evaluated as rootstocks for the bell pepper hybrids Margarita (Syngenta) and Pampa (Clause) as scions, under natural infestation of *Ralstonia pseudosolanacearum*, as well as upon artificial inoculation with a) ten *Ralstonia* isolates, b) one *Phytophthora capsici* isolate, c) *Meloidogyne incognita* race 1, and d) *Meloidogyne enterolobii* (only rootstocks for c and d). Grafted bell peppers consistently showcased a higher productivity than did non-grafted ones under natural *R. pseudosolanacearum* infestation, and the rootstock genotype affected fruit size distribution. Rootstocks presented higher resistance than did commercial bell pepper hybrids (scions) for 6 out of 10 *Ralstonia* isolates. AF-8253 and BRS Acará displayed an immune-like response to *P. capsici*, while Fortaleza was moderately resistant (25% symptomatic plants) and the bell pepper hybrids (scions) were susceptible (100% symptomatic plants). Rootstocks were immune to *M. incognita* race 1, but slightly (AF-8253 and BRS Acará) or moderately (Fortaleza) resistant to *M. enterolobii*. Grafting with BRS Acará, as well as with AF-8253 or Fortaleza, is recommended for managing bacterial wilt, Phytophthora blight, and root-knot nematodes.

Keywords: *Capsicum annuum* L.; grafting; *Meloidogyne*; *Phytophthora capsici*; *Ralstonia*.

INTRODUCTION

Bell pepper (*Capsicum annuum* L.) is one of the most important vegetable crops grown in tropical and subtropical regions (Onoyama *et al.*, 2010; Wang *et al.*, 2018), generating good revenue for producers around the world (Pimenta *et al.*, 2016). Its production in greenhouses has consistently increased in Brazil and, without adequate crop rotation, the problems with soilborne pathogens have increased too. Bell peppers are especially vulnerable to the following soilborne pathogens: the bacterial species complex of *Ralstonia* genus, the oomycete *Phytophthora capsici*, and the nematodes belonging to the genus *Meloidogyne* (Guerrero *et al.*, 2014; Pinheiro *et al.*, 2014; Soares *et al.*, 2018).

The *Ralstonia* species complex includes various pathogenic races, biovars, phylotypes, and sequevars (Fegan & Prior, 2005) causing bacterial wilt of *Capsicum*. Bacterial wilt of bell peppers in Brazil is predominantly caused by race 1, biovar 3, phylotype I (Lopes & Boiteux, 2004; Santiago *et al.*, 2020), which has been recently reclassified as a new species, named *Ralstonia pseudosolanacearum* (Rossato *et al.*, 2018; Santiago *et al.*, 2020).

Phytophthora capsici causes Phytophthora blight and is considered the most destructive soilborne pathogen in *Capsicum* crops worldwide (Gómez-Rodríguez *et al.*, 2017). It causes root rot and stem blight, as well as several secondary symptoms, such as sudden leaf wilting with successive plant defoliation, fruit necrosis, plant tipping over, and death (Dunn *et al.*, 2014; Barchenger *et al.*, 2018).

The root-knot nematode (*Meloidogyne* spp.) is also a soilborne pathogen that is highly harmful to bell peppers (Pinheiro *et al.*, 2014). In addition to the formation of galls

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that impair water and nutrient absorption, the damaged roots become sites of infection by fungi and bacteria, enhancing damages to the crop (Mota *et al.*, 2013). Over 95 species are described in the genus *Meloidogyne* (Ghule *et al.*, 2014), but *M. incognita* (Kofoid & White), *M. javanica* (Treub), *M. arenaria* (Neal) Chitwood, and *M. hapla* Chitwood are the ones that cause the greatest impact on bell pepper production (Wang *et al.*, 2018). Recently, *M. enterolobii* has gained importance as cultivating plants resistant to the major *Meloidogyne* species is proving inadequate for controlling this particular species (Pinheiro *et al.*, 2014).

Using genetic resistance is the best strategy for managing soilborne diseases. Resistance gene pyramiding in rootstocks used for grafting is useful for overcoming such diseases, since no resistant commercial bell pepper hybrids are available (Mihajloviæ *et al.*, 2017; Barchenger *et al.*, 2018). Several rootstocks can be found on the Brazilian market, including AF-8253 (Sakata Seed Sudamerica), which is advertised by the seed company as having a high level of resistance to *P. capsici* and the *Ralstonia* complex, as well as the nematodes *M. javanica* and *M. incognita,* races 1, 2, 3, and 4; and Fortaleza (Takii Seed), recommended by its producing company for cultivation in areas with bacterial wilt, Phytophthora blight, and root-knot nematode infestation. Moreover, the *Capsicum* breeding program of the Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária, Embrapa) released a new hybrid rootstock, BRS Acará, with multiple disease resistance and a high potential for success in the market. Previous studies have indicated satisfactory compatibility of this rootstock with some bell pepper hybrids, namely Margarita (Syngenta), Rubia R (Sakata Seed Sudamerica), Magali R (Sakata Seed Sudamerica), and Maximos (Clause) (Madeira *et al.*, 2016).

This research compared Embrapa's hybrid rootstock BRS Acará with AF-8253 and Fortaleza by grafting them to two major bell pepper hybrids used in Brazil, Margarita (Syngenta) and Pampa (Clause), and inoculating them with *Ralstonia* complex, *P. capsici*, *M. incognita*, and *M. enterolobii*.

MATERIAL AND METHODS

An experiment under natural infestation with *R. pseudosolanacearum*, as well as three experiments under artificial inoculation with *Ralstonia* spp., *P. capsici*, *M. incognita* and *M. enterolobii*, were carried out at Embrapa Hortaliças, Brasília, DF, Brazil.

Reaction to *R. pseudosolanacearum* in a naturally infested soil

Bell pepper hybrids Margarita (Syngenta Crop Protection, Greensboro, United States) and Pampa (HM.CLAUSE Inc, Davis, United States) studied as scions grafted onto the commercial rootstocks AF-8253 (Sakata Seeds Sudamerica, Bragança Paulista, Brazil), Fortaleza (Takii Seed, Kyoto, Japan), and BRS Acará (Embrapa, Brasília, Brazil), as well as the non-grafted hybrids (scions without any rootstock), were cultivated in a greenhouse with soil naturally infested with *R. pseudosolanacearum*. The pathogen was identified by biochemical tests and multiplex PCR analysis as *Ralstonia pseudosolanacearum* race 1, biovar 3, phylotype I of the *Ralstonia* complex (Safni *et al.*, 2014). All genotypes were sown in July 2016, in polystyrene trays using commercial substrate. Whenever required, the cleft grafting method was used, with the aid of a scalpel and a clamp.

One experiment was carried out, during spring 2016 and summer 2017. We used a factorial scheme 2 x 3 + 2 (two bell pepper cultivars × three rootstock hybrids + two non-grafted bell pepper cultivars) in a randomized complete block design, with six replications and plots with ten plants in double rows. The soil was classified as Typic Hapludox, pH 5.75, with the following composition: 529.7 mg dm^{-3} P, 73 mg dm^{-3} K, 5.5 cmol$_c$ dm^{-3} Ca, 3.3 cmol$_c$ dm^{-3} Mg, 0.0 cmol$_c$ dm^{-3} Al, and 26.0 g dm^{-3} organic matter (O.M.). Fertilization was carried out with 150 kg ha^{-1} N applied as urea, 200 kg ha^{-1} P_2O_5 applied as single superphosphate, 2.2 kg ha^{-1} B applied as borax, and 4 kg ha^{-1} Zn applied as zinc sulfate. Seedlings were transplanted to the plots ten days after grafting, with a separation of 0.4 m between plants in the same row and 0.4 m between rows in a bed of two rows, while the spacing between two beds (two double-lines) was 1.6 m. Plants were drip irrigated and tutored. Fertigation was performed biweekly with ammonium sulfate and mono potassium phosphate (MKP) until the last harvest, corresponding to a total of 200 kg ha^{-1} N, 150 kg ha^{-1} P_2O_5, and 200 kg ha^{-1} K_2O.

Six harvests were carried out between November 2016 and March 2017. Fruits were classified according to the length classes adopted by the Brazilian market: large (> 15 cm), medium (12 to 15 cm), and small (8 to 12 cm). Fruits damaged or smaller than 8 cm were considered only for calculating the number and productivity of total fruits (NTF and PTF, respectively). The sum of large, medium, and small fruits was recorded as number and productivity of marketable fruits (NMF and PMF, respectively). The data of the PTF and the PMF per hectare, and of NTF and NMF in percentage of fruits in size-classes were analyzed with an analysis of variance and the Scott & Knott test ($p < 0.05$).

Reaction to soilborne pathogens upon artificial inoculation Ralstonia spp.

The bell pepper hybrids Margarita and Pampa (scions), as well as the rootstocks BRS Acará, Fortaleza, and AF-

8253, were inoculated with four *Ralstonia* spp. isolates (2017 experiment) and seven *Ralstonia* spp. isolates (2018 experiment). All five genotypes (two scion bell peppers and three rootstock hybrids) were sown in polystyrene trays filled with commercial substrate. Seedlings were inoculated with one of the isolates of *Ralstonia* spp. by spraying the roots (plugs containing the roots) of each plant with 5 mL of a bacterial suspension containing approximately 10^8 CFU mL^{-1} (Lopes & Boiteux, 2016) right after the seedlings were detached from trays for transplanting. Transplanting was carried out 47 days after sowing (DAS) to 0.5 L plastic pots with sterile soil mixture kept in a greenhouse with night heating used to prevent temperature drop below 20°C that could increase the chance of escapes. During the experimental period, the temperature was 30°±10°C. The greenhouse experiment was arranged in a completely randomized design, with three replications and six plants per plot (one plant per pot) in 2017 and four replications and four plants per plot in 2018 (one plant per pot), in a factorial scheme *Capsicum* genotypes x *Ralstonia* spp. isolates.

The isolates used in 2017 were three *R. pseudosolanacearum* CNPH RS594, CNPH RS628 and CNPH RS639, respectively originated from Sergipe, Amazonas and Pará States, Brazil, and one *R. solanacearum* biovar I, isolate CNPH RS652, from São Paulo State, Brazil. Seven isolates were used in 2018, four being *R. pseudosolanacearum*, CNPH RS541, CNPH RS634, CNPH RS672 and CNPH RS639 respectively originated from Federal District, Piauí, Amazonas and Pará States, Brazil, and three being *R. solanacearum* biovar I, isolates CNPH RS668, CNPH RS623 and CNPH RS670, the first one being originated from Espírito Santo, and the others from São Paulo State, Brazil.

Disease severity was assessed 15 days after inoculation (DAI; 62 DAS). The scores ranged from 1 to 5, the lowest grade corresponding to the total absence of wilting and the highest one corresponding to the plant death. Plants with irreversible wilting symptoms were attributed grades equivalent to or above 3. Scores from 1.5 to 2.5 were given to plants with a light wilting symptom, which could recover following irrigation.

Phytophthora capsici

Seedlings of all five genotypes were transplanted 47 DAS to 1.0 L pots with sterile soil mixture, two plants per pot. Two days after transplanting, the base of each plant was poured with 3 mL of a solution containing 2 × 10^4 zoospores mL^{-1} of the *P. capsici* isolate Pcp 116. The Pcp 116 isolate belongs to *P. capsici* race 18; it was collected in a *Capsicum baccatum* field in Goiás State, Brazil. A completely randomized design was used, with five treatments, four replications, and two plants per plot. Disease incidence was evaluated by the index of plants with symptoms (%), attributed to each plot 13 DAI (62 DAS). The score attributed to each plot (0, 50, or 100) corresponded to the percentage of symptomatic plants. Wilted plants with a darkened stem base, which is typical of the disease, were considered as symptomatic plants.

Root-knot nematodes

Seedlings of the rootstocks were cultivated in pots under controlled conditions before being inoculated with the root-knot nematodes *M. incognita* race 1 or *M. enterolobii*. Seedlings were transplanted 70 DAS to 2.0 L pots, filled with sterile substrate composed of soil, washed sand, cattle manure, and carbonized rice straw in equal volume parts. Following transplanting, the plants were inoculated with a 5.0 mL suspension containing 5,000 eggs and second-stage juveniles (J2) of *M. incognita* race 1 or *M. enterolobii*. A completely randomized design was used, with six replications consisting of one plant each. Treatments consisted of the three rootstock hybrids (‘AF-8253’, ‘Fortaleza’ and ‘BRS Acará’), a resistant control for *M. incognita* (i.e., tomato cultivar Nemadoro) and a susceptible control (i.e., tomato cultivar Rutgers).

The traits evaluated were the egg mass index (EMI), gall index (GI), number of eggs and second-stage juveniles per gram of root (NERG), reproduction factor (RF) (Oostenbrink, 1966), and reproduction index (RI%) (Taylor, 1967; Soares *et al.*, 2018) 70 days after inoculation (DAI). The indexes EMI and GI were evaluated to facilitate the interpretation of the NERG, RF, and RI. Plants were collected separately, the roots were washed in running water, and egg masses were colored according to Dickson & Struble (1965). Then, the number of egg masses in each root system was calculated under a stereoscopic microscope. The EMI was estimated according to Huang *et al.* (1986), using grades 1 to 5. The GI was determined by grades 1 to 5, according to Charchar *et al.* (2003), galls bigger than 3 mm being considered as large. For the NERG analysis, roots were washed, dried at room temperature (15° - 30 C), and weighed, before being processed according to Hussey & Barker (1973), modified by Boneti & Ferraz (1981). The RF was determined by dividing the final (Pf) and initial (Pi) population densities ($RF = \frac{Pf}{Pi}$) (Oostenbrink, 1966). Plants with RF = 0 were considered as immune (I), plants with RF < 1 as resistant (R), and plants with RF > 1 as susceptible (S). The RI% was obtained by dividing the plot’s Pf by the Pf of the susceptible control (tomato cultivar Rutgers). Plants with RI > 50% were considered as susceptible, plants with RI between 26% and 50% as slightly resistant, between 11% and 25% as moderately resistant, between 1% and 10% as very resistant, plants with RI < 1% as highly resistant, and plants with RI=0% as immune (Taylor, 1967; Soares *et al.*, 2018).

Statistical analyses for the artificial inoculation experiments were carried out and means were grouped with the Scott & Knott test ($p < 0.05$).

RESULTS AND DISCUSSION

Production in a soil naturally infested with *R. pseudosolanacearum*

The incidence of bacterial wilt influenced decisively the yield and quality of fruits. Wilting symptom was evenly distributed through the experimental area and grafting strongly reduced the occurrence of wilting. Non-grafted Margarita was the most affected genotype (30% wilted plants), whereas the wilt incidence in Margarita was reduced to 8.3%, 3.3%, and 0% when grafted onto Fortaleza, BRS Acará, and AF-8253, respectively.

The production of the grafted treatments was compared directly to their respective non-grafted control using a line-contrast analysis (Table 1), aiming to test the hypothesis that grafting was advantageous for cultivation in a soil infested with *R. pseudosolanacearum*. The factorial experiment (rootstocks × bell pepper hybrids) was analyzed excluding non-grafted controls, which allowed testing differences, as well as the interaction, between rootstocks and bell pepper hybrids.

For all production variables, a difference between at least one grafted treatment and its respective non-grafted control was noticed. Higher values were consistently found for the grafted treatments, not only confirming the effectiveness of this technique for overcoming bacterial wilt, but also showing that this advantage depends on the rootstock genotype.

The fruit mass in the smallest marketable size class (8–12 cm) was lower for Margarita grafted onto AF-8253 (41%) than for non-grafted Margarita (54%). Margarita grafted onto Fortaleza and BRS Acará, as well as non-grafted Margarita, averaged 53% of fruits with a length of 8–12 cm. Percentages of Margarita fruits in the other size classes were 43% for 12–15 cm and 2% for fruits longer than 15 cm. Pampa produced the same percentage of fruits with a length of 12–15 cm (47%) regardless it was grafted or not. Nevertheless, the percentage of fruits longer than 15 cm decreased when Pampa was grafted (namely 2%, 1.7%, and 0.5% when grafted onto BRS Acará, AF-8253, and Fortaleza, respectively) in comparison to non-grafted Pampa (7.8%). Finally, the percentage of 8–12 cm long Pampa fruits was higher for grafted (54%, 49%, and 42% for Fortaleza, BRS Acará, and AF-8253, respectively) than non-grafted Pampa (37%).

Productivity was compared between non-grafted controls with a contrast analysis and differences were significant ($p < 0.05$). Pampa presented a higher yield than Margarita, resisting more under *R. pseudosolanacearum* infestation, which was probably due to a higher level of resistance (Table 1).

Concerning the factorial analysis (rootstock × bell pepper hybrid), a significant effect of the bell pepper hybrid on the number of total and marketable fruits per hectare was observed. Pampa presented a higher number of fruits per hectare (462,100 total and 418,000 marketable fruits ha^{-1}) than did Margarita (422,300 total and 372,300 marketable fruits ha^{-1}). The total number and number of marketable fruits per hectare (442,200 and 395,100 fruits ha^{-1}, respectively) were not affected by the rootstock, and the total mass of fruits (59.9 t ha^{-1}) and the mass of marketable fruits per hectare (57.1 t ha^{-1}) were not affected by rootstock or bell pepper hybrid.

Although the rootstock genotype did not influence the productivity variables, it did affect fruit-size distribution (Table 2). Nevertheless, the percentage of marketable fruits (total of marketable classes, in mass) was not affected by the rootstock or bell pepper genotype (average of 95%).

Table 1: Total and marketable fruit yields of bell pepper hybrids Margarita and Pampa, both grafted and non-grafted, from a greenhouse naturally infested with *Ralstonia pseudosolanacearum*. Brasília, 2017

		Total yield (per hectare)				Marketable yield (per hectare)			
		Number of fruits (thousands)		Mass (t)		Number of fruits (thousands)		Mass (t)	
	HYBRID	Margarita	Pampa	Margarita	Pampa	Margarita	Pampa	Margarita	Pampa
ROOTSTOCK	Non-grafted	326.8	395.4	37.43	50.25	260.3	362.2	35.72	48.54
	AF-8253	419.4**	464.2**	60.91**	56.64^{ns}	378.6**	421.8^{ns}	58.35**	54.05^{ns}
	Fortaleza	419.1**	436.4^{ns}	55.03**	64.54*	370.6**	385.2^{ns}	52.23*	61.40*
	BRS Acará	428.4**	485.7**	61.52**	60.58^{ns}	367.7**	446.9*	57.96**	58.61^{ns}
	C.V. (%)	9.94^{++}		18.33^{++}		15.16^{++}		20.08^{++}	

^{ns}non-significant, *significant ($p < 0.05$) and **highly significant ($p < 0.01$) difference between a given grafting treatment and its corresponding non-grafted control; $^{++}$highly significant ($p < 0.01$) effect of treatments (hybrids and rootstocks) in the analysis of variance.

Reaction to soilborne pathogens upon artificial inoculation *Ralstonia* species complex

The genotypes (rootstock and scion) reacted differently to the *Ralstonia* isolates. Symptoms were not observed for the isolate CNPH RS623, thus indicating its low virulence. This was probably due to long time preservation *in vitro*. As for the isolates CNPH RS628 and CNPH RS652, typical wilting was observed (Figures 1 and 2). The interaction between the genotypes and the *Ralstonia* isolates was significant, indicating that the level of resistance of a genotype depends on the isolate, as suggested by Lopes & Boiteux (2004).

All rootstocks displayed an immune-like response to CNPH RS594 and CNPH RS634. Similar results were observed for CNPH RS668, but the difference was not significant of the rootstocks in comparison to the bell pepper Margarita. Overall, the rootstock genotypes presented a significantly higher resistance to the isolates

Table 2: Commercial size class distribution of fruits produced by bell pepper hybrids grafted onto different rootstocks under *Ralstonia pseudosolanacearum* natural infestation

	Size class	> 15 cm (%[1])		]12; 15] cm	]8; 12] cm
	HYBRID	**Margarita**	**Pampa**	(%[1])	
ROOTSTOCK	**AF-8253**	5.0[a]	1.5	50.9[a]	41.4[b]
	Fortaleza	0.2[b]	0.5	40.9[b]	53.3[a]
	BRS Acará	0.5[b]	2.0	42.9[b]	50.9[a]
	C.V. (%)	82.4**[; 2]	82.4[ns; 2]	21.9*	18.6**

[1]percentage was calculated in mass; [2]coefficient of variation of data transformed into cubic root; [ns]non-significant, *significant ($p < 0.05$) and **highly significant ($p < 0.01$) differences among rootstocks.

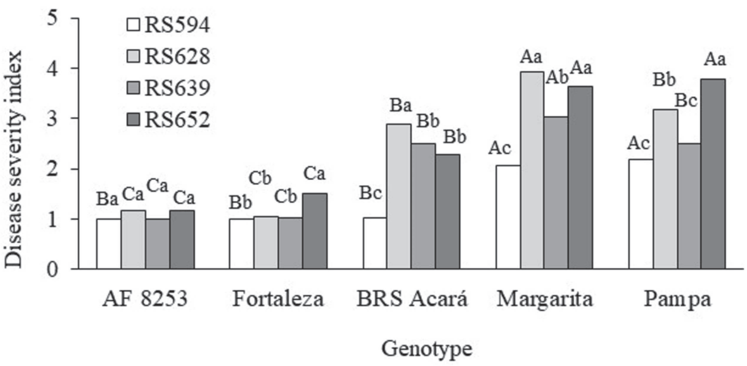

Figure 1: Severity of wilt caused by four *Ralstonia* isolates (RS) from Embrapa Hortaliças bank (CNPH) in bell pepper hybrids and rootstock genotypes, evaluated in 2017 through scores ranging from 1 (no wilt) to 5 (completely dried leaves). Different uppercase letters indicate different scores among genotypes considering a *Ralstonia* isolate and distinct lowercase letters indicate different virulence of *Ralstonia* isolates for a plant genotype, as assessed by the Scott & Knott test (p < 0.05), CV 12.9%.

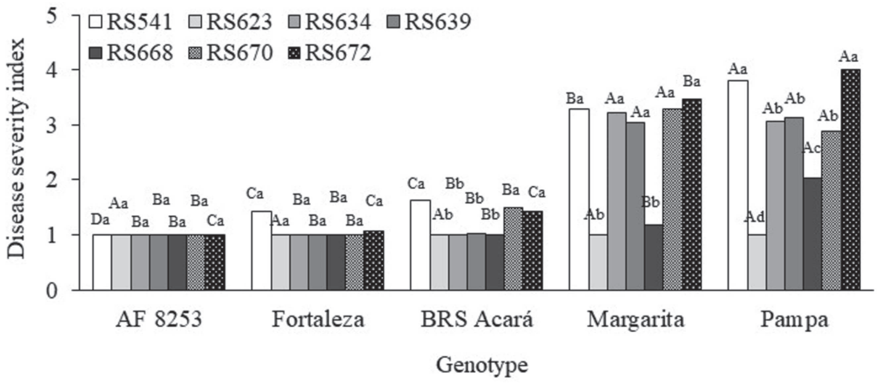

Figure 2: Severity of wilt caused by seven *Ralstonia* isolates (RS) from Embrapa Hortaliças bank (CNPH) in bell pepper and rootstock genotypes, evaluated in 2018 through scores ranging from 1 (no wilt) to 5 (completely dried leaves). Different uppercase letters indicate different scores among genotypes considering a *Ralstonia* isolate (RS) and distinct lowercase letters indicate different virulence of *Ralstonia* isolates for a plant genotype, as assessed by the Scott & Knott test (p < 0.05), CV 20.6%.

CNPH RS541, CNPH RS594, CNPH RS634, CNPH RS652, CNPH RS670, and CNPH RS672, than did the bell pepper hybrids. Individually, the rootstock genotypes AF-8253, Fortaleza, and BRS Acará were more resistant than the bell pepper hybrids, namely for eight, eight, and six *Ralstonia* isolates out of the ten isolates evaluated, respectively.

There were instances of similar resistance levels between bell pepper hybrids and rootstock genotypes for three isolates (CNPH RS623, CNPH RS668, and CNPH RS628). That is, the bell pepper hybrid Margarita was similarly resistant to the isolate CNPH RS668 as were all the three rootstock genotypes, whereas the hybrid Pampa and the rootstock BRS Acará had comparable resistance levels to the isolate CNPH RS628. However, none of the bell pepper hybrids showcased a resistance level higher than that of any of the rootstocks. This reinforces the advantage of using those rootstocks as a strategy to face bacterial wilt in bell pepper crops.

The five genotypes did not react differently between *R. solanacearum* and *R. pseudosolanacearum*. Although *R. pseudosolanacearum* has been found to be more aggressive to *Capsicum* spp. (Lopes *et al.*, 2015), the isolates CNPH RS652 and CNPH RS670 (*R. solanacearum*) were highly virulent too. This fact reinforces the statement from Lopes & Boiteux (2016) that resistance to bacterial wilt is isolate-specific rather than species or phylotype (or biovar) specific. In this way, breeding for resistance must be undertaken with local *Ralstonia* isolates.

We emphasize that *Capsicum* spp. have not been considered as susceptible hosts of *Ralstonia* spp., unlike *Solanum* species, especially potatoes and tomatoes. Therefore, choosing a rootstock should consider the prevalence of aggressive strains at the location and the contamination level (bacterial population) in the soil. Under normal conditions in Southern and Southeastern Brazil, where *R. solanacearum* is prevalent, we hypothesize that an intermediate level of resistance would be enough to protect against bacterial wilt under crop rotation cultivation. However, higher resistance levels would be necessary for the disease-conducive climate of Northern and Northeastern Brazil, whose regions are characterized by the prevalence of *R. pseudosolanacearum*.

Phytophthora capsici

The genotypes presented different levels of resistance to *P. capsici* (Figure 3). Margarita and Pampa were not resistant to *P. capsici* or, at least, to the isolate used in this study. Contrastingly, all studied rootstocks had a higher resistance level in comparison to the commercial bell pepper hybrids, thus underlining the advantages of grafting. Notably, the best grafting results for controlling *P. capsici* are likely to be obtained using AF-8253 or BRS Acará rather than Fortaleza because it has not displayed an immune-like reaction to *P. capsici* isolate Pcp 116 as did AF-8253 and BRS Acará.

Most *Capsicum* cultivars are either very susceptible or only partially resistant to *P. capsici* (Barchenger *et al.*, 2018). The resistant phenotypes are determined by a single dominant gene for each *P. capsici* race in *C. annuum* (Monroy-Barbosa & Bosland, 2010). Thus, plant breeders must pyramid multiple resistance genes in a cultivar to generate host resistance to several diseases and for the cultivar to survive to each single race of *P. capsici* (Barchenger *et al.*, 2018). Ribeiro & Bosland (2012) reported the high virulence of race 18 in pepper genotypes, the same race used in this research, which highlights the importance of the immunity of BRS Acará and AF-8253.

Root-knot nematodes

Both *M. incognita* race 1 and *M. enterolobii* population increased considerably in the susceptible control, the tomato cultivar Rutgers (Table 3).

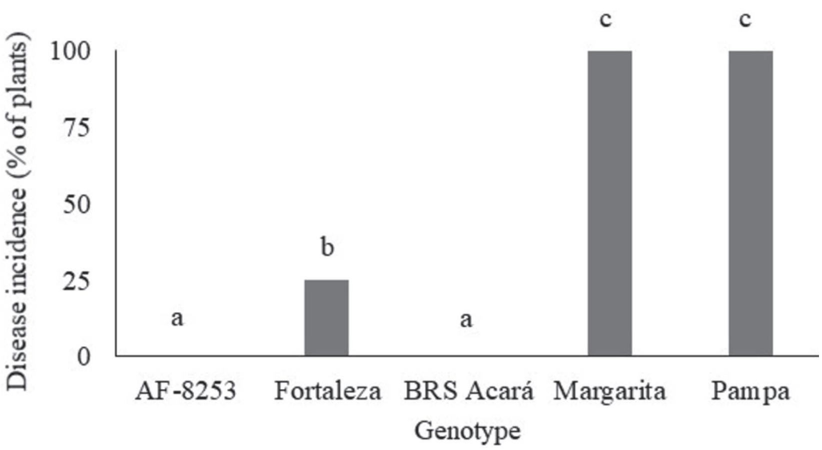

Figure 3: Incidence of wilt caused by *Phytophthora capsici* in bell pepper and rootstock genotypes. Different letters indicate significant difference, as assessed by the Scott & Knott test ($p < 0.05$), CV 28.69%.

Table 3: Reaction of rootstocks and control genotypes to root-knot nematodes *Meloidogyne incognita* and *Meloidogyne enterolobii*

Genotype	*Meloidogyne incognita* race 1				Reaction (RF)	RI (%)[6]	Reaction (RI)
	EMI[1]	GI[2]	NERG[3]	RF[4]			
Nemadoro	1.0	1.0	67.2^{b}	0.3^{b}	Resistant	1.26^{b}	Very resistant
Rutgers	5.0	4.7	7486.7^{a}	30.7^{a}	Susceptible	100.00^{a}	Susceptible
AF-8253	1.3	1.3	21.2^{c}	0.0^{c}	Resistant	0.27^{c}	Highly resistant
Fortaleza	1.0	1.0	20.5^{c}	0.0^{c}	Resistant	0.22^{c}	Highly resistant
BRS Acará	1.3	1.3	18.8^{c}	0.0^{c}	Resistant	0.13^{c}	Highly resistant
CV (%)	16.89	21.43	31.20**	35.08**		30.08**	

Genotype	*Meloidogyne enterolobii*				Reaction	RI (%)[5]	Reaction (RI)
	EMI[1]	GI[2]	NERG[5]	RF[5]			
Nemadoro	4.7	5.0	2889.8^{b}	22.5^{a}	Susceptible	109.70^{a}	Susceptible
Rutgers	5.0	5.0	4879.3^{a}	20.3^{a}	Susceptible	100.00^{a}	Susceptible
AF-8253	5.0	5.0	2905.8^{b}	5.8^{b}	Susceptible	29.88^{b}	Slightly resistant
Fortaleza	4.0	4.0	1543.5^{b}	3.0^{b}	Susceptible	14.36^{c}	Moderately resistant
BRS Acará	4.8	4.8	4702.8^{a}	6.7^{b}	Susceptible	32.03^{b}	Slightly resistant
CV (%)	12.16	10.97	28.13*	23.61**		21.34**	

[1]egg mass index; [2]gall index; [3]number of eggs and second-stage juveniles per root gram, coefficient of variation and statistical analysis used data transformed by *sen* $(x + 1)$; [4]reproduction factor, coefficient of variation and statistical analysis used data transformed by $1/(x + 0.17)$; [5]reproduction index, coefficient of variation and statistical analysis used data transformed with $\sqrt[3]{x}$; [6]coefficient of variation and statistical analysis used data transformed with, coefficient of variation and statistical analysis used data transformed with ; *significant ($p < 0.05$) and **highly significant ($p < 0.01$) difference between genotypes.

The resistance of the tomato cultivar Nemadoro to *M. incognita* race 1 was confirmed and the auxiliary variables EMI and GI, in general, confirmed the results obtained for NERG, RF, and RI for both nematode species. All rootstocks were immune or highly resistant to *M. incognita* race 1, based on the RF or RI values, respectively. Contrastingly, all the rootstocks were classified as susceptible to *M. enterolobii* according to their RF, while slightly resistant (AF-8253 and BRS Acará) or moderately resistant (Fortaleza) according to their RI. Interestingly, the Scott-Knott grouping of RF coincided with the RI classification into different levels of resistance or susceptibility.

Resistance has been reported in some lines and cultivars of *Capsicum* to different *Meloidogyne* species and races (Hendy *et al.*, 1985; Fery *et al.*, 1998; Djian-Caporalino *et al.*, 1999; Castagnone-Sereno *et al.*, 2001; Thies & Fery, 2002). Possibly, the studied rootstocks carry one or more major pepper resistance genes to *M. incognita* (Djian-Caporalino *et al.*, 2011).

No genotype was resistant to *M. enterolobii* according to the RF. One rootstock genotype was classified as moderately resistant and the other two were slightly resistant based on the RI. Although the reference (susceptible control) used to calculate the RI in our study was a tomato (instead of *Capsicum* spp.) cultivar, known to be highly susceptible, the obtained RI showed the actual response of the rootstocks to *M. enterolobii*. That is, the classification confirmed the formation of multiple groups of suscetibility.

Meloidogyne enterolobii is an emerging pathogen and few sources of resistance in the genus *Capsicum* are described in the literature (Pinheiro *et al.*, 2020). Moreover, preliminary studies have shown that *Capsicum* peppers are more susceptible to *M. enterolobii* than to other species of root-knot nematodes (Pinheiro *et al.*, 2020).

Melo *et al.* (2011) reported a moderate resistance to *M. enterolobii* in two *Capsicum* genotypes, namely BGH-433 and BGH-4285, in keeping with the lower susceptibility of the *Capsicum* rootstocks studied herein. Moreover, reports on different levels of susceptibility or even resistance in *Capsicum* can be found in the literature (Pinheiro *et al.*, 2013). Most recently, Pinheiro *et al.* (2020) reported resistance to *M. enterolobii* in BRS Nandaia, a habanero pepper cultivar of the *C. chinense* species.

CONCLUSIONS

Grafting with all the rootstock hybrids evaluated in this study was equally effective for commercial production in soil naturally infested with *R. pseudosolanacearum*. Moreover, the rootstocks presented significantly higher resistance to most of the *Ralstonia* spp. isolates than did the bell pepper hybrids.

Two of the rootstocks, AF-8253 and BRS Acará, displayed an immune-like response to *P. capsici*, while Fortaleza was moderately resistant, and both the bell pepper hybrids Margarita and Pampa (scions) were highly susceptible to this pathogen.

All three rootstocks were immune to *M. incognita* race 1; Fortaleza was moderately resistant, and AF-8253 and BRS Acará were slightly resistant to *M. enterolobii*.

Grafting with BRS Acará, as well as with AF-8253 and Fortaleza, is recommended for managing *Ralstonia*, *P. capsici*, and the root-knot nematodes *M. incognita* race 1 and *M. enterolobii*.

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